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Durable Complete Responses in Heavily Pretreated Patients with Metastatic Melanoma Using T Cell Transfer Immunotherapy

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Abstract

Purpose—Most treatments for patients with metastatic melanoma have a low rate of complete regression and thus overall survival in these patients is poor. We have investigated the ability of adoptive cell transfer utilizing autologous, tumor infiltrating lymphocytes to mediate durable complete regressions in heavily pre-treated patients with metastatic melanoma.

Experimental Design—Ninety-three patients with measurable metastatic melanoma were treated with the adoptive transfer of autologous tumor-infiltrating lymphocytes administered in conjunction with interleukin-2 following a lymphodepleting preparative regimen on three sequential clinical trials. Ninety-five percent of these patients had progressive disease following a prior systemic treatment. Median potential followup was 62 months.

Results—Objective response rates by RECIST criteria in the three trials using lymphodepleting preparative regimens (chemotherapy alone or with 2Gy or 12Gy irradiation) were 49%, 52% and 72%. Twenty of the 93 patients (22%) achieved a complete tumor regression and 19 have ongoing complete regressions beyond three years The actuarial three and five year survivals for the entire group were 36% and 29% respectively but for the 20 complete responders were 100% and 93%. The likelihood of achieving a complete response was similar regardless of prior therapy. Factors associated with objective response included longer telomeres of the infused cells, the number of CD8+ CD27+ cells infused and the persistence of the infused cells in the circulation at one month (all $p_2<0.001$).

Conclusions—Cell transfer therapy with autologous tumor infiltrating can mediate durable complete responses in patients with metastatic melanoma and has similar efficacy irrespective of prior treatment.

Introduction

Patients with metastatic melanoma have a poor prognosis with a 5 year survival rate of about 5%[1]. There are two FDA approved treatments for these patients. Dacarbazine has an objective response rate of approximately 12% with 2–3 % complete responses that are often

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transient[2]. Interleukin-2 (IL-2) has an objective response rate of approximately 15% with 4–5% durable complete responses[3]. Results of two new experimental agents have recently been reported for the treatment of patients with this disease. Ipilimumab, an antibody against the inhibitory lymphocyte receptor, CTLA4, mediated a 3.6 month improvement in median survival with an objective response rate of 7% in 540 patients but only three patients (0.6%) achieved a complete regression[4]. PLX4032, an inhibitor of mutated BRAF, had an objective response rate of 77% in 48 patients with 3 (6%) complete regressions[5]. The very small number of durable complete responses make it unlikely that many patients with metastatic melanoma will be cured utilizing any of these approaches.

There are several advantages to the use of lymphocyte transfer as an immune-based approach to treat cancer[6]. Large numbers of lymphocytes can be selected in vitro for high reactivity against tumor antigens and grown under conditions that overcome the tolerizing influences that exist in vivo. Perhaps most importantly it is possible to modify the host prior to the cell transfer to eliminate immune regulatory cells and provide an optimal microenvironment for the transferred cells. Prior studies have shown that transfer of cultured lymphocytes with anti-viral activity can prevent cytomegalovirus[7] and Epstein-Barr virus (EBV) infections and the subsequent development of post-transplant lymphoproliferative diseases[8]. Fresh and cultured lymphocytes have been used to treat relapsed leukemias and lymphomas after allogeneic bone marrow transplantation as well as established EBV induced lymphomas and nasopharyngeal tumors[8–14]. A patient with metastatic melanoma responding to the transfer of cloned CD4 lymphocytes has been reported[15].

We have previously reported early results in patients with metastatic melanoma treated with the adoptive transfer of autologous tumor infiltrating lymphocytes (TIL) selected for anti-tumor activity, expanded in vitro and reinfused into patients along with IL-2 following a lymphodepleting preparative regimen[16, 17]. We are now reporting the definitive analysis of this series with a median potential follow-up of 62 months in 93 patients with metastatic melanoma. These patients all had progressive disease and were heavily pretreated with standard as well as experimental regimens. Of the 93 treated patients, 52 (56%) had an objective response. Twenty patients (22%) experienced a complete regression, 19 of whom have ongoing complete responses beyond three years. It thus appears that for this group of heavily pretreated patients for whom a lesion can be resected to obtain TIL, and who received the infusion, a high rate of complete, and possibly curative regressions can be achieved.

Methods

Patients

Patients were eligible for these trials if they were 18 years of age or older, with measurable metastatic melanoma and Eastern Cooperative Oncology Group (ECOG) performance status of zero or one, life expectancy of greater than 3 months, and no evidence of active systemic infections, coagulation disorders or other active major medical or cardiovascular or immunodeficiency diseases. Patients had progressive metastatic melanoma at entrance into the protocol and at least 4 weeks had elapsed after any prior treatment. All patients had a metastatic lesion of greater than 2 cm diameter that could be resected for growth of TIL and had cells that grew in culture to sufficient quantities with in vitro anti-tumor activity as described below. Patients with one or two brain metastases less than 1 cm in diameter were eligible for this trial. All patients signed an informed consent approved by the Institutional Review Board of the National Cancer Institute.

Clinical Trial Design

Prior to receiving TIL infusion, all patients received a non-myeloblative lymphodepleting regimen consisting of cyclophosphamide at 60mg/kg/d for two days, and fludarabine at 25 mg/m2/d for five days. In three sequential trials, patients received this non-myeloblative preparative regimen alone (43 patients) or in conjunction with total body irradiation (TBI) either 2 Gy (25 patients) or 12 Gy (2 Gy twice a day for 3 days; 25 patients). TBI was delivered at 2 Gy fractions with 15-MV photons delivered at a distance of six meters to the patient's midline at a dose rate of 0.11 Gy per minute as previously described[17]. Within a day following the completion of the preparative regimen patients received an intravenous infusion of TIL and were started on high-dose IL-2 at 720,000 IU/kg intravenously every 8 hours to tolerance. Within one or two days after TIL infusion patients who received TBI also received a minimum of 2×10^6/kg of autologous purified cryopreserved CD34+ hematopoetic stem cells from a granulocyte colony stimulating factor mobilized pheresis. Patient response to treatment was assessed utilizing Response Evaluation Criteria in Solid Tumors (RECIST) guidelines starting at approximately 4 weeks after cell administration and at regular intervals thereafter. Data in this report are updated as of August 1, 2010 with a median potential followup of 89.8, 58.5 and 41.5 months in the cohorts with preparative chemotherapy alone, or with 2 or 12 Gy respectively (overall median potential followup 62.0 months; range 35.1 to 118.6 months).

Preparation of Tumor Infiltrating Lymphocytes for Infusion

Lymphocytes were grown from resected metastatic melanoma lesions in high-dose IL-2 as previously described[16–18]. In brief, individual cultures in 24 well culture plates were established from either single cell suspensions or 1–2 mm^3 fragments. The wells were individually grown and expanded and tested for the presence of antigen specificity utilizing overnight cytokine release coculture assays against either autologous tumor or HLA matched melanoma cell lines. Cultures with evidence of specific reactivity compared to allogeneic non-MHC matched controls that exceeded 200 pg/ml of interferon gamma and were at least twice control values were selected for rapid expansion as previously described[16, 17].

The average length of telomere repeats in chromosomes from the infused cells was measured by quantitative flow-FISH as previously described[19]. The number and percent of CD8+ CD27+ cells in the infused TIL was measured after withdrawal of IL-2 for two days[20]. The persistence of the infused cells in the circulation was measured by amplifying TCR BV region sequences using the SMART RACE cDNA Amplification kit (Clontech Laboratories, Inc., Mountain View, CA) and comparing clonotypes in the infused TIL with those in the circulation at one month using techniques previously described[21, 22].

Statistical Analysis

Continuous parameters were compared between response groups (CR vs. <CR; PR+CR vs. NR) using an exact Wilcoxon rank sum test since the majority of parameters were not normally distributed in one or both of the groups to be compared. An exact Cochran-Armitage test was used to compare ordered categorical parameters (degree of TBI: 0, 200, 1200) and stage (M1a, M1b, and M1c) with response group[23]. Dichotomous parameters (sex, HLA 02 vs. non-02) were compared with response group using Fisher's exact test. All p-values are two-tailed and presented without adjustment for multiple comparisons. In view of the number of parameters initially explored (12 for each response group comparison) p-values <0.01 would likely indicate significant associations while those for which $0.01 < p < 0.05$ would indicate strong trends.

Results

There were no significant differences in sex, age, ECOG status or the number of cells administered among the three treatment cohorts (chemotherapy preparative regimen alone or plus 2 Gy or 12 Gy). The incidence and duration of responses in each of the three treatment cohorts are presented in Table 1 and patient survival in Figure 1. The objective response rates in the three cohorts were 49%, 52% and 72% respectively with complete response rates of 12%, 20%, and 40%. There was no significant difference in overall response rate among the cohorts ($p_2 = 0.08$) though there was an increased rate of complete responses associated with increasing dose of TBI ($p_2=0.007$; Table 2) (results are descriptive and not corrected for multiple comparisons). These cohorts were accrued sequentially and thus should be compared to each other with caution. The actuarial 3 and 5 year survivals for the entire 93 patients were 36% and 29% respectively. For the 20 complete responders the 3 and 5 year survivals were 100% and 93%, for the 32 partial responders 31% and 21% and for the 41 nonresponders 7% and 5%.

Of particular interest was the high incidence of durable complete responses seen in 20 of the 93 patients (22%) with complete regressions ongoing in 19 of these patients at 37 to 82 months. All but two of the completely responding patients received a single treatment; two received a second treatment. Eighty of the 93 patients (86%) had visceral metastases (stages M1b or M1c) including 17 of the 20 (85%) complete responders and 28 of 32 (88%) partial responders. Complete responses were seen in patients with a median of 3 different organ sites of metastases including lung, liver, adrenal, muscle, lymph nodes and skin. The exact sites of disease in patients in the three cohorts are shown in Supplementary Table 1 and the stage of metastatic disease in each cohort with their response is shown in Supplementary Table 2. Representative scans and photos of the 20 patients who experienced a complete response are shown in Supplementary Figure 1. Although 19 of the patients who experienced a complete response are ongoing long-term survivors, 8 additional patients also are alive beyond 3 years, 6 of whom had prolonged partial responses (4 had all residual disease resected), one had a mixed response and the other responded to subsequent chemotherapy.

This was a heavily pretreated group of patients as shown in Table 3. Seventy-seven of the 93 patients (83%) had progressed after receiving IL-2, either at high dose or as part of a biochemotherapy regimen, 40 (43%) had prior chemotherapy and 37 (40%) had received both IL-2 and chemotherapy. Of the 20 complete responders, 70% had received prior IL-2, 35% prior chemotherapy, 30% both IL-2 and chemotherapy, 55% prior interferon and 25% prior anti-CTLA4. The median time from first diagnosis of metastatic disease to the administration of cell therapy was 18 months (mean ± 25.9 ± 2.7 months). The median number of prior systemic treatments administered was 2 (mean ± SEM 2.0 ± 0.2). The five year survival of 29% for all 93 patients was similar regardless of prior treatment with the possible exception of the 44% five year survival of the 11 patients that progressed after receiving prior anti-CTLA4 (Figure 3). These latter 11 patients had received anti-CTLA4 an average of 13.7 months before starting the cell transfer therapy and all had progressive disease.

There were no significant differences in patients who experienced a complete response or any objective response compared to non-responders in sex, age, HLA type, metastatic stage, numbers of cells administered or LDH labels (Table 2). However, patients experiencing an objective response received fewer IL-2 doses than non-responders (7.2 ± 0.3 vs. 8.8 ± 0.4; $p_2 = 0.003$), likely due to increased side effects from IL-2 resulting from increased activity of the transferred cells in responders that limited IL-2 dosing in vivo. As shown in Table 2 and Figure 2 objective responders differed significantly from non-responders in receiving

TIL with longer average telomeres, a larger number of CD8+ CD27+ cells and in the in vivo persistence (of the infused cells) in the circulation at one month (all $p_2 < 0.001$). There was considerable overlap between responders and nonresponders for each of these three factors and thus none absolutely predicted the occurrence of an objective response. There was no difference in response rates in patients with tumors that did or did not contain BRAF or NRAS mutations.

It should be emphasized that the patients in this trial were a select group who had a resectable lesion (estimated to be about 85% of all patients). In our entire experience, viable TIL could be grown to a minimum of 5×10^6 cells (and the majority to over 2×10^7 cells) from 75 to 85% of patients depending on whether the cultures were set up as enzymatic digests, fragments or both. Active specific TIL were identified in 67% of all resected patients[24].

The toxicities of treatment have been previously reported and were largely due to the lymphodepleting preparative regimen or IL-2[17, 25]. Most patients tolerated the treatment well and returned to baseline. There was one treatment-related death in the 93 patients; a 49 year old male who received 2 Gy TBI and died of sepsis four days after cell infusion from an undetected diverticular abcess present prior to treatment. One patient with a partial response who received 2Gy TBI developed prolonged pulmonary hypertension. Five patients who received 12 Gy TBI, all of whom experienced a complete regression developed a microangiopathic nephropathy with creatinine elevations in the range of 1.5 to 2.5 mg/dL. Renal function has not worsened over time and these patients are living normally.

Discussion

Although many systemic treatments for patients with metastatic solid cancers can mediate modest improvements in survival, the long-term cure of patients requires the induction of durable complete regressions. With few exceptions (such as men with germ cell tumors or women with choriocarcinoma) durable complete regressions of metastatic solid cancers are very rare. In patients with metastatic melanoma the administration of high dose IL-2 can mediate durable complete apparently curative responses in 4 to 5% of patients and this led to the FDA approval of IL-2 for patients with melanoma in 1998[3, 26, 27]. Durable complete regressions using dacarbazine, the only other FDA approved treatment for melanoma, is seen in less than 1% of patients[1, 2]. Recent reports of the use of ipilimumab, a molecule reactive with CTLA4 on the cell surface in 540 patients[4] and PLX4032, a mutated BRAF inhibitor in 48 patients[5] have demonstrated complete responses in 0.6% and 6% of patients with metastatic melanoma respectively, though both treatments appear capable of prolonging the survival of patients.

In the current report, durable complete responses were seen in 22% of patients who received this cell transfer therapy and 95% of these complete responses are ongoing beyond three years. It thus appears to be an effective and possibly curative treatment for many patients with metastatic melanoma capable of receiving it.

Patients with melanoma have been the subject of many attempts at immunotherapy[6]. Early reports of a low level of objective responses of melanoma to immunologic modulation with IL-2[28] and the demonstration that lymphocytes infiltrating into melanomas (TIL) specifically recognized tumor-associated antigens[29] suggested that this disease stimulated an endogenous immune response that could be further manipulated to improve anti-tumor effects. Although the immunization of patients with cancer antigens (cancer vaccines) has thus far yielded only modest results[30], early studies of the isolation, growth in IL-2 and infusion of autologous TIL demonstrated that this adoptive immunotherapy approach could

mediate transient tumor regression in patients though the lack of persistence of the transferred cells may have hampered their effectiveness[31, 32]. An improvement of this treatment reported in 2002 demonstrated that chemotherapy-induced lymphodepletion prior to adoptive cell infusion could lead to the dramatic enhancement of the persistence of the transferred cells and improved anti-cancer effects[16]. Studies in tumor-bearing mice demonstrated that the anti-tumor effects of adoptive cell transfer were a direct function of the magnitude of lymphodepletion[33] and these findings led to studies of the addition of total body irradiation to the nonmyleoablative chemotherapy preparative regimen that had been used in earlier trials. Earlier reports of these pilot trials showed that 10 of 93 patients achieved a complete regression though follow-up in the trials using maximum lymphodepletion at the time of that report was only 10 months[17]. We now report the definitive long-term follow-up of these trials utilizing adoptive transfer of autologous TIL following preparative lymphodepleting treatment with an overall median potential follow-up of 62 months and a median potential follow-up of 42 months in the latest cohort adding 12 Gy irradiation. Ten of the partial responders have now converted to complete durable regressions without any further treatment. This is likely due to the resolution of scarring after tumor destruction or to the ongoing anti-tumor impact of persisting T lymphocytes for months after cell transfer. Of the 93 patients, 20 (22%) have achieved a complete regression of metastatic disease and 19 of these 20 patients have ongoing complete responses beyond three years. Regressions have been seen at all visceral sites. The average patient had a median of three different metastatic anatomic sites.

Of particular importance is the induction of durable complete responses in patients who have received and progressed through multiple prior treatment regimens. Seventy percent of the complete responders had progressed through IL-2, 35% through chemotherapy and 30% had both IL-2 and chemotherapy. Of the 11 patients that had previously progressed after receiving anti-CTLA4 monoclonal antibody, five experienced a complete regression in addition to two partial regressions. There was no influence of any prior therapy on the likelihood of achieving a complete regression or on overall survival in these 93 patients suggesting that this treatment approach can be useful as an upfront treatment or as a salvage regimen for patients with progression after other therapies.

Although complete regressions were seen in patients receiving each of the preparative regimens there is a strong suggestion that increasing the lymphodepletion by adding TBI enhanced the anti-tumor effects. Studies in murine models suggested that lymphodepletion prior to adoptive cell transfer reduced the competition for homeostatic cytokines such as IL-7 and IL-15[34] that promote lymphocyte growth and indeed we measured an increase in serum IL-15 in all patients on the day after the lymphodepleting regimen was completed[17]. Other impacts of the lymphodepletion are likely due to transient elimination of regulatory T cells and enhancement of the activity of antigen presenting cells[35, 36]. In preliminary studies we have seen an inverse correlation between the likelihood of response and the return of $CD4^+Foxp3^+$ cells in the circulation after treatment and this is now being further studied. In concert with results from murine models indicating that infused cells that were less differentiated and with a higher proliferative potential had increased anti-tumor activity[37] we noted a highly significant association between the likelihood of having a complete response and the infusion of TIL with longer telomeres, TIL with more $CD8^+$ $CD27^+$ cells and increased persistence in the circulation of the infused cells at one month after transfer. There is an inverse correlation of telomere length with time in culture[38] though there was no maximum time in culture that precluded administration of cells. These findings point the way towards increasing the therapeutic effectiveness of TIL by choosing cultures with higher average telomere lengths or cultures containing cells with a higher absolute number of $CD27^+$ cells, a marker of less differentiated cells[37]. Generation of less-differentiated cells might also be accomplished by minimizing time in culture and use of alternative cytokines.

It should be emphasized that not all patients with metastatic melanoma can receive this treatment approach. A metastatic nodule of at least 2 cm diameter must be present and suitable for resection. Most resections have been from soft tissue lesions but peripheral lung and liver lesions have also been used[24]. Metastatic lesions resected for symptomatic relief in the course of disease progression or lymph nodes resected for Stage III disease can be cryopreserved and are a suitable source for later growth of TIL. Since about 85% of patients have lesions capable of being resected and about 55% grow cells suitable for infusion we estimate that about 45% of all patients with metastatic melanoma can receive this treatment. About 5% of patients develop complications of tumor growth during the 4 to 6 weeks of cell preparation that preclude treatment. An important advantage of this cell transfer approach is its ability to mediate complete regressions regardless of the failure of prior treatments.

Current efforts are devoted to developing simpler and faster methods to grow TIL with increased anti-tumor efficacy and to develop alternative preparative regimens. TIL grown by a simplified method that eliminates in vitro testing for anti-tumor activity[38] have mediated tumor regressions in patients with melanoma, though followup in these patients is short[39, 40]. The ability to transduce genes encoding anti-tumor T cell receptors into normal circulating lymphocytes and the use of these genetically engineered autologous cells for adoptive transfer is being explored in ongoing clinical trials[41, 42].

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

References

1. Balch CM, Gershenwald JE, Soong S, et al. Final version of 2009 AJCC melanoma staging and classification. J Clin Oncol. 2009; 27:6199–6206. [PubMed: 19917835]
2. Middleton MR, Grob JJ, Aaronson N, et al. Randomized phase III study of temozolomide versus dacarbazine in the treatment of patients with advanced metastatic malignant melanoma. J Clin Oncol. 2000; 18:158–166. [PubMed: 10623706]
3. Atkins MB, Lotze MT, Dutcher JP, et al. High-dose recombinant interleukin-2 therapy for patients with metastatic melanoma: analysis of 270 patients treated between 1985 and 1993. J Clin Oncol. 1999; 17:2105–2116. [PubMed: 10561265]
4. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. N Engl J Med. 2010; 363:711–723. [PubMed: 20525992]
5. Flaherty KT, Puzanov I, Kim KB, et al. Inhibition of mutated, activated BRAF in metastatic melanoma. N Engl J Med. 2010; 363:809–819. [PubMed: 20818844]
6. Rosenberg SA, Restifo NP, Yang JC, Morgan RA, Dudley ME. Adoptive cell transfer: a clinical path to effective cancer immunotherapy. Nat Rev Cancer. 2008; 8:299–308. [PubMed: 18354418]
7. Walter EA, Greenberg PD, Gilbert MJ, et al. Reconstitution of cellular immunity against cytomegalovirus in recipients of allogeneic bone marrow by transfer of T-cell clones from the donor. N Engl J Med. 1995; 333:1038–1044. [PubMed: 7675046]
8. Rooney CM, Smith CA, Ng CY, et al. Infusion of cytotoxic T cells for the prevention and treatment of Epstein-Barr virus-induced lymphoma in allogeneic transplant recipients. Blood. 1998; 92:1549–1555. [PubMed: 9716582]
9. Kolb HJ, Mittermuller J, Clemm C, et al. Donor leukocyte transfusions for treatment of recurrent chronic myelogenous leukemia in marrow transplant patients. Blood. 1990; 76(12):2462–2465. [PubMed: 2265242]
10. Mackinnon S, Papadopoulos EB, Carabasi MH, et al. Adoptive immunotherapy evaluating escalating doses of donor leukocytes for relapse of chronic myeloid leukemia after bone marrow transplantation: Separation of graft-versus-leukemia responses from graft-versus-host disease. Blood. 1995; 86:1261–1268. [PubMed: 7632930]

11. Khanna R, Bell S, Sherritt M, et al. Activation and adoptive transfer of Epstein-Barr virus-specific cytotoxic T cells in solid organ transplant patients with posttransplant lymphoproliferative disease. Proc Natl Acad Sci,USA. 1999; 96:10391–10396. [PubMed: 10468618]
12. Straathof K, Bollard C, Popat U, et al. Treatment of nasopharyngeal carcinoma with Epstein-Barr virus-specific T lymphocytes. Blood. 2005; 105:1898–1904. [PubMed: 15542583]
13. Comoli P, Pedrazzoli P, Maccario R, et al. Cell therapy of stage IV nasopharyngeal carcinoma with autologous Epstein-Barr virus-targeted cytotoxic T lymphocytes. J Clin Oncol. 2005; 23:8942–8949. [PubMed: 16204009]
14. Bollard C, Aguilar L, Straathof K, et al. Cytotoxic T lymphocyte therapy for Epstein-Barr virus Hodgkin's disease. J Exp Med. 2004; 200:1623–1633. [PubMed: 15611290]
15. Hunder NN, Wallen H, Cao J, et al. Treatment of metastatic melanoma with autologous CD4+ T cells against NY-ESO-1. N Engl J Med. 2008; 358:2698–2703. [PubMed: 18565862]
16. Dudley ME, Wunderlich JR, Robbins PF, et al. Cancer regression and autoimmunity in patients after clonal repopulation with anti-tumor lymphocytes. Science. 2002; 298:850–854. [PubMed: 12242449]
17. Dudley ME, Yang JC, Sherry R, et al. Adoptive cell therapy for patients with metastatic melanoma: Evaluation of intensive myeloablative chemoradiation preparative regimens. J Clin Oncol. 2008; 26:5233–5239. [PubMed: 18809613]
18. Dudley ME, Wunderlich JR, Shelton TE, Even J, Rosenberg SA. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. J Immunother. 2003; 26:332–342. [PubMed: 12843795]
19. Zhou J, Shen X, Hodes RJ, Rosenberg SA, Robbins P. Telomere length of transferred lymphocytes correlates with in vivo persistence and tumor regression in melanoma patients receiving cell transfer therapy. J Immunol. 2005; 175:7046–7052. [PubMed: 16272366]
20. Huang J, Kerstann KW, Ahmadzadeh M, et al. Modulation by IL-2 of CD70 and CD27 expression on CD8+ T cells: importance for the therapeutic effectiveness of cell transfer immunotherapy. J Immunol. 2006; 176:7726–7735. [PubMed: 16751420]
21. Huang J, El-Gamil M, Dudley ME, Li YF, Rosenberg SA, Robbins PF. T cells associated with tumor regression recognize frameshifted products of the CDKN2A tumor suppressor gene locus and a mutated HLA class I gene product. J Immunol. 2004; 172(6057):6064.
22. Robbins PF, Dudley ME, Wunderlich J, et al. Cutting edge: Persistence of transferred lymphocyte clonotypes correlates with cancer regression in patients receiving cell transfer therapy. J Immunol. 2004; 173:7125–7130. [PubMed: 15585832]
23. Agresti, A. Categorical Data Analysis. New York: John Wiley and Sons, Inc.; 1990.
24. Goff SL, Smith FO, Klapper JA, et al. Tumor infiltrating lymphocytes (TIL) therapy for metastatic melanoma: Analysis of tumors resected for TIL. J Immunother. 2010; 33:840–847. [PubMed: 20842052]
25. Dudley ME, Wunderlich JR, Yang JC, et al. Adoptive cell transfer therapy following non-myeloablative but lymphodepleting chemotherapy for the treatment of patients with refractory metastatic melanoma. J Clin Oncol. 2005; 23:2346–2357. [PubMed: 15800326]
26. Rosenberg SA, Yang JC, Topalian SL, et al. Treatment of 283 consecutive patients with metastatic melanoma or renal cell cancer using high-dose bolus interleukin-2. JAMA. 1994; 271:907–913. [PubMed: 8120958]
27. Smith FO, Downey SG, Klapper JA, et al. Treatment of metastatic melanoma using interleukin-2 alone or in conjunction with vaccines. Clin Cancer Res. 2008; 14:5610–5618. [PubMed: 18765555]
28. Rosenberg SA, Lotze MT, Muul LM, et al. Observations on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. N Engl J Med. 1985; 313:1485–1492. [PubMed: 3903508]
29. Muul LM, Spiess PJ, Director EP, Rosenberg SA. Identification of specific cytolytic immune responses against autologous tumor in humans bearing malignant melanoma. J Immunol. 1987; 138:989–995. [PubMed: 3100623]
30. Rosenberg SA, Yang JC, Restifo NP. Cancer immunotherapy: moving beyond current vaccines. Nat Med. 2004; 10:909–915. [PubMed: 15340416]

31. Rosenberg SA, Packard BS, Aebersold PM, et al. Use of tumor infiltrating lymphocytes and interleukin-2 in the immunotherapy of patients with metastatic melanoma. Preliminary report. N Engl J Med. 1988; 319:1676–1680. [PubMed: 3264384]
32. Rosenberg SA, Yannelli JR, Yang JC, et al. Treatment of patients with metastatic melanoma using autologous tumor-infiltrating lymphocytes and interleukin-2. J Natl Cancer Inst. 1994; 86:1159–1166. [PubMed: 8028037]
33. Wrzesiniski C, Paulos CM, Kaiser A, et al. Increased intensity lymphodepletion enhances tumor treatment efficacy of adoptively transferred tumor-specific T cells. J Immunother. 2010; 33:1–7. [PubMed: 19952961]
34. Gattinoni L, Finkelstein SE, Klebanoff CA, et al. Removal of homeostatic cytokine sinks by lymphodepletion enhances the efficacy of adoptively transferred tumor-specific CD8+ T cells. J Exp Med. 2005; 202:907–912. [PubMed: 16203864]
35. Antony PA, Piccirillo CA, Akpinarli A, et al. CD8+ T cell immunity against a tumor/self-antigen is augmented by CD4+ T helper cells and hindered by naturally occurring T regulatory cells. J Immunol. 2005; 174:2591–2601. [PubMed: 15728465]
36. Gattinoni L, Powell DJ, Rosenberg SA, Restifo NP. Adoptive immunotherapy for cancer: building on success. Nat Rev Immunol. 2006; 6:383–393. [PubMed: 16622476]
37. Gattinoni L, Klebanoff CA, Palmer DC, et al. Acquisition of full effector function in vitro paradoxically impairs the in vivo antitumor efficacy of adoptively transferred CD8+ T cells. J Clin Invest. 2005; 115:1616–1626. [PubMed: 15931392]
38. Tran KQ, Zhou J, Durflinger KH, et al. Minimally cultured tumor-infiltrating lymphocytes display optimal characteristics for adoptive cell therapy. J Immunother. 2008; 31:742–751. [PubMed: 18779745]
39. Besser MJ, Shapira-Frommer R, Treves AJ, et al. Clinical responses in a phase II study using adoptive transfer of short-term cultured tumor infiltration lymphocytes in metastatic melanoma patients. Clin Cancer Res. 2010; 16:2646–2655. [PubMed: 20406835]
40. Dudley ME, Gross CA, Langhan MM, et al. CD8+ enriched "young" tumor infiltrating lymphocytes can mediate regression of metastatic melanoma. Clin Cancer Res. 2010; 16:6122–6131. [PubMed: 20668005]
41. Morgan RA, Dudley ME, Wunderlich JR, et al. Cancer regression in patients after transfer of genetically engineered lymphocytes. Science. 2006; 314:126–129. [PubMed: 16946036]
42. Johnson LA, Morgan RA, Dudley ME, et al. Gene therapy with human and mouse T-cell receptors mediates cancer regression and targets normal tissues expressing cognate antigen. Blood. 2009; 114:535–546. [PubMed: 19451549]

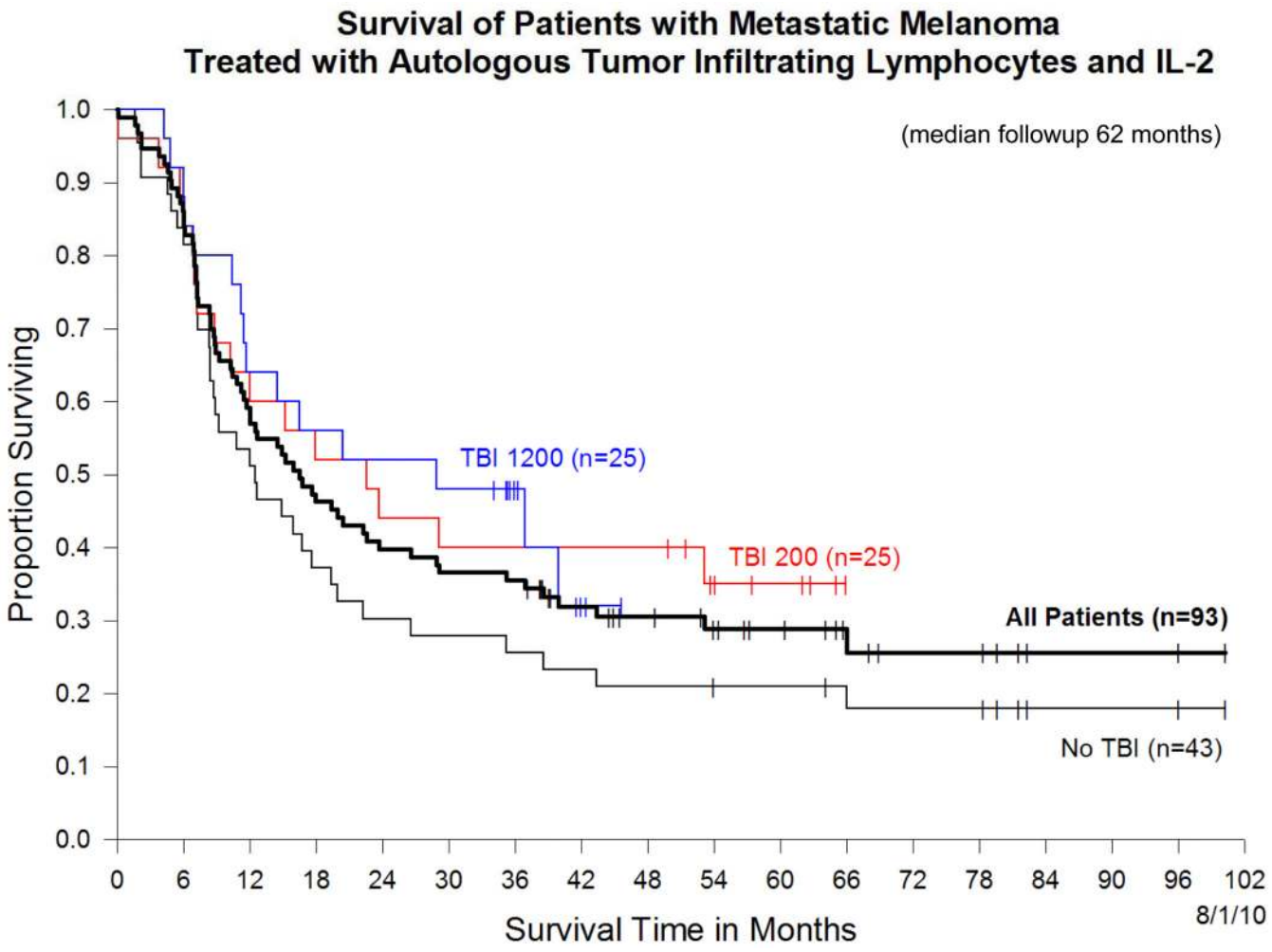

Figure 1.
Overall survival of patients receiving TIL with the chemotherapy preparative regimen alone (No TBI) or plus 2 Gy or 12 Gy total body irradiation.

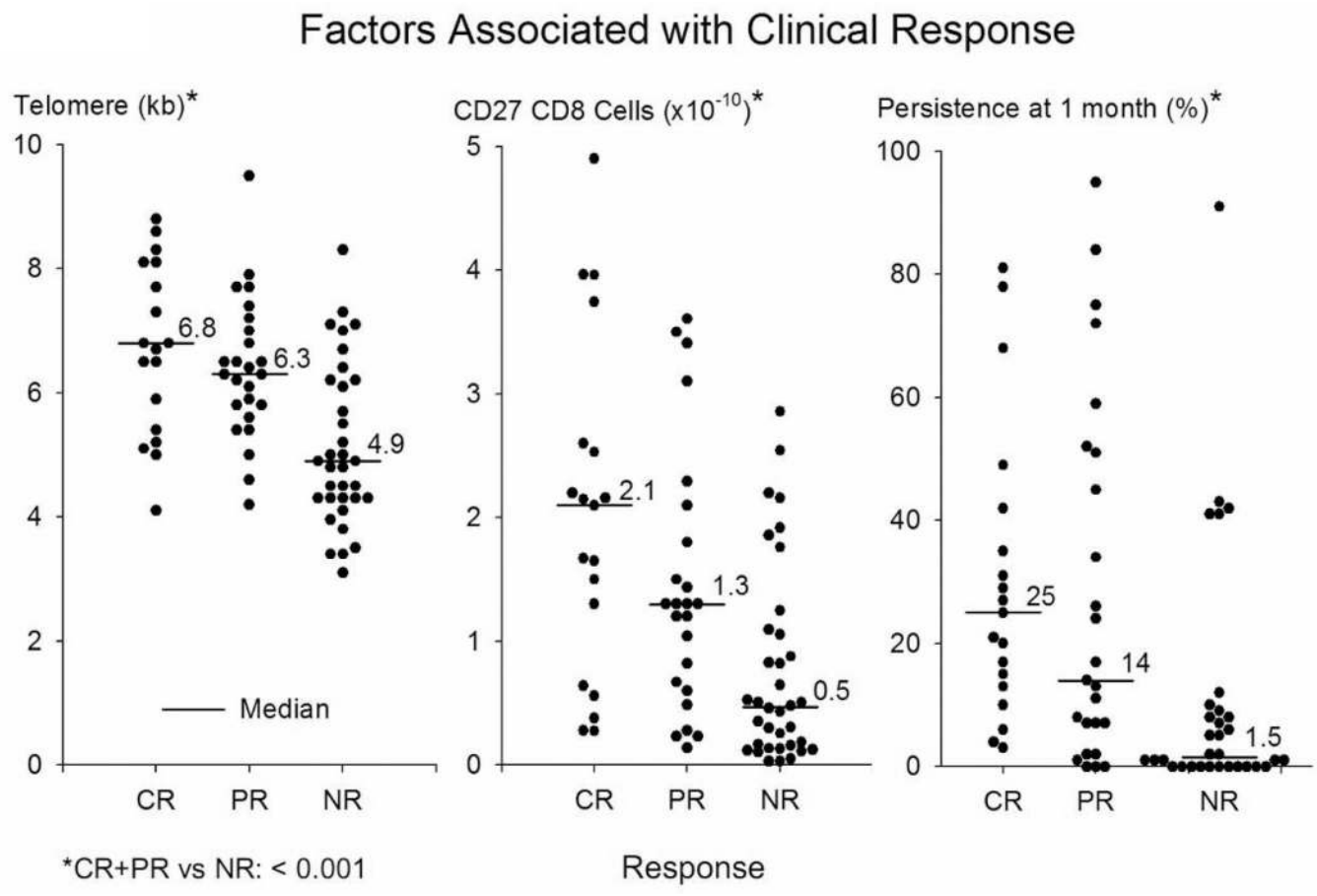

Figure 2.

Mean telomere length, the number of CD27+ CD8+ cells and the percent persistence of the infused cells in peripheral blood at one month after cell infusion are significantly different in objective responders (CR + PR) compared to non-responders (all $p_2 < 0.001$).

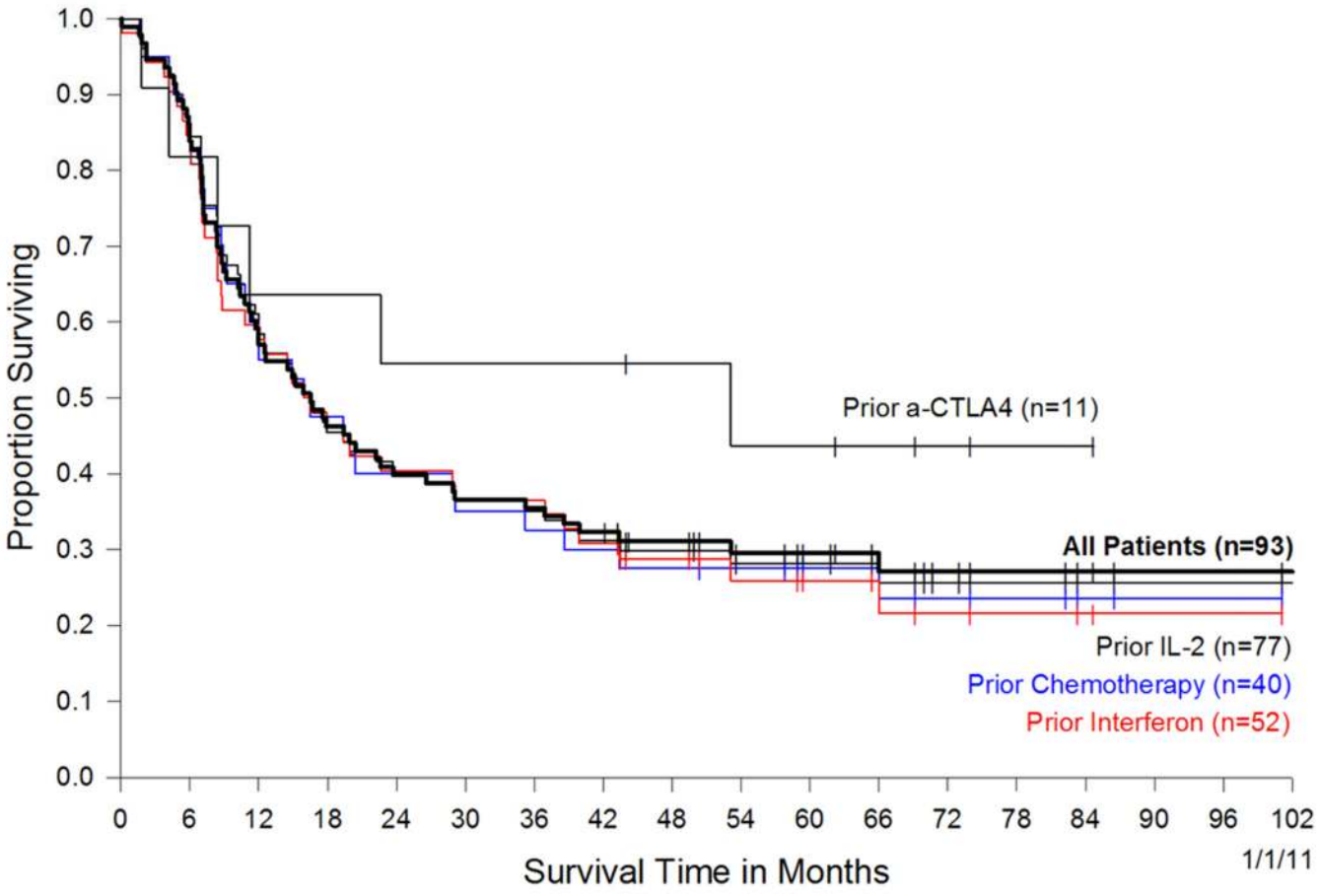

Figure 3.
Overall survival of patients receiving cell transfer based on prior treatment received.

Table 1

CELL TRANSFER THERAPY

Treatment	Total	PR	CR	OR (%)
		number of patients (duration in months)		
No TBI	43	16 (37%) (84, 36, 29, 28, 14, 12, 11, 7, 7, 7, 7, 4, 4, 2, 2, 2)	5 (12%) (82+, 81+, 79+, 78+, 64+)	21 (49%)
200 TBI	25	8 (32%) (14, 9, 6, 6, 5, 4, 3, 3)	5 (20%) (68+, 64+, 60+, 57+, 54+)	13 (52%)
1200TBI	25	8 (32%) (21, 13, 7, 6, 6, 5, 3, 2)	10 (40%) (48+, 45+, 44+, 44+, 39+, 38+, 38+, 38+, 37+, 19)	18 (72%)
TOTAL	93	32 (34%)	20 (22%)	52 (56%)

Table 2

PATIENT AND TREATMENT CHARACTERISTICS

		(number of patients)					
		CR*	**PR***	**NR***	**Total**	**CR vs (PR+NR)**	**(CR+PR) vs NR**
						(p_2)	
Total		20	32	41	93		
Patients							
Sex	Male	13	20	29	62	1.0	<0.51
	Female	7	12	12	31		
Age	16 – 30	3	5	4	12	0.84	0.79
	31 – 45	7	11	14	32		
	46 – 60	10	16	21	47		
	61 – 75	0	0	2	2		
HLA	A2	15	28	32	75	0.53	0.61
	non-A2	5	4	9	18		
Stage**	M1a	3	4	6	13	0.55	0.56
	M1b	3	4	1	8		
	M1c	14	24	34	72		
Treatments							
TBI (Gy)	0	5	16	22	43	0.007	0.08
	2	5	8	12	25		
	12	10	8	7	25		
Cells ($\times 10^{-10}$)	<3	4	5	10	19		
	3.1 – 5.0	3	4	12	19		
	5.1 – 7.0	7	12	7	26		
	7.1 – 9.0	2	4	4	10		
	>9	4	7	8	19		
	mean ± SEM	6.5 ± 0.7	6.1 ± 0.5	5.5 ± 0.6		0.25	0.07
	% $CD3CD8^+$	86 ± 2	79 ± 4	74 ± 4		0.59	0.14
	% $CD3CD4^+$	10 ± 2	20 ± 4	24 ± 4		0.30	0.15
Telomere length (kb)		6.7 ± 0.3	6.4 ± 0.2	5.1 ± 0.2		0.006	<0.001

		(number of patients)					
		CR*	**PR***	**NR***	**Total**	**CR vs (PR+NR)**	**(CR+PR) vs NR**
CD8+ CD27+ cells ($\times 10^{-10}$)		2.0 ± 0.3	1.5 ± 0.2	0.8 ± 0.1		0.001	<0.001
Persistence at 1 month (%)		30.2 ± 5.5	28 ± 5.9	10.5 ± 3.5		0.003	<0.001
IL-2 doses	<5	1	3	1	5		
	5 – 8	15	22	19	56		
	9 – 11	4	7	21	32		
	mean ± SEM	7.1±0.4	7.7±0.5	8.8±0.4		0.11	0.003

*CR, complete response; PR partial response; NR no response

**M1a, skin, subcutaneous or nodal metastases; M1b lung; M1c all other visceral sites or elevated lactic dehydrogenase

Table 3

IMPACT OF PRIOR TREATMENT ON RESPONSE TO CELL TRANSFER THERAPY USING SELECTED TIL

	Total	CR number (%)*	PR	OR**
All Patients Prior Treatment	93	20(22%)	32(34%)	52(56%)
None	5(5%)	2(40%)	1(20%)	3(60%)
IL-2	77(83%)	14(18%)	28(36%)	42(54%)
Chemotherapy	40(43%)	7(18%)	16(40%)	23(58%)
Interferon	52(56%)	11(21%)	17(33%)	28(54%)
Anti-CTLA4	11(12%)	5(45%)	2(18%)	7(64%)
IL-2+ Chemotherapy	37(40%)	6(16%)	16(43%)	22(59%)
IL-2+ Anti-CTLA4	8(9%)	3(38%)	1(13%)	4(50%)
IL-2+ Anti-CTLA4+ Chemotherapy	6(7%)	2(33%)	1(17%)	3(50%)

*This refers to the percent of patients with a CR, PR or OR in each group that had received the prior treatment.

**No group is statistically different from any other